Quanterix™
The Science of Precision Health

Infectious Disease

Simoa® SARS-CoV-2 N Protein Antigen Test Sensitivity

When evaluating relative performance characteristics for SARS-CoV-2 diagnostic tests, it is important to understand how different measures of sensitivity are established. There are two distinct characteristics that are evaluated by the FDA for all tests receiving Emergency Use Authorization that demonstrate the test's ability to accurately and robustly detect positive subjects: analytical sensitivity, and clinical sensitivity.

Analytical Sensitivity, or Limit of Detection (LoD):

To enable a common methodology between disparate SARS-CoV-2 diagnostic test types, the LoD is reported in units of TCID50/mL (or Median Tissue Culture Infectious Dose/mL). These studies are typically conducted with inactivated virus from a commercial supplier or public repository, which determines the initial TCID50/mL as the virus concentration at which 50% of cells are determined as being infected by displaying cytopathic effects. The limit of detection for a diagnostic test is defined as the lowest viral concentration at which at least 19 of 20 replicate samples are identified as positive by the test. The test LoD is not subject to the sources of experimental variability that may confound the determination of clinical sensitivity as discussed below, and thus represents a meaningful basis for comparison between tests. As shown in Table 1, excerpted directly from the FDA website, the Simoa SARS-CoV-2 N Protein Antigen Test has a reported LoD of 0.29 TCID50/mL, corresponding to an analytical sensitivity >100-fold greater than any other SARS-CoV-2 Antigen test currently authorized for Emergency Use by the FDA.

Table 1. Comparison table for SARS-CoV-2 Antigen Tests Authorized for Emergency Use.*

NP	Matrix	PPA	NPA	LoD(TDIC50/mL)	Simoa Advantage	# Study Subjects
Quanterix Simoa	**NP**	**96%**	**100%**	**0.29**		**898**
Celltrion Sampinute	NP	94%	100%	30	103-fold	72
LumiraDx	NP & Nasal	98%	97%	32	110-fold	257
Celltrion DiaTrust	NP	93%	99%	32	110-fold	133
Luminostics	Nasal Swab	97%	100%	88	303-fold	166
Sofia 2 Flu	NP & Nasal	95%	100%	91.7	316-fold	164
BD Veritor	Nasal Swab	84%	100%	140	483-fold	226
Abbot BinaxNOW	Nasal Swab	85%	99%	140.6	485-fold	460
Qorvo Omnia	Nasal Swab	90%	100%	200	690-fold	89
Quidel Sofia	NP & Nasal	97%	100%	226	779-fold	209
BD Veritor Flu Combo	Nasal Swab	87%	100%	280	966-fold	278
Diasorin Liason	NP	96%	99%	300	1034-fold	185
Diasorin Liason	Nasal Swab	97%	100%	300	1034-fold	141
Ortho Vitrios	NP & Nasal	86%	98%	500	1724-fold	152
CareStart | Access Bio	NP	94%	99%	800	2759-fold	180
CareStart | Access Bio	Nasal Swab	87%	100%	800	2759-fold	92
Salofa Oy Sienna Clarity	NP	88%	99%	1250	4310-fold	133
Status Flu Combo	NP	94%	100%	2700	9310-fold	125
InBios SCov2 Ag Detect	Nasal Swab	87%	100%	6300	21724-fold	302
Ellume	Nasal Swab	95%	97%	6309	21755-fold	198
Quidel QuickVue	Nasal Swab	84%	99%	19100	65862-fold	161

*Source: FDA Website: Antigen Tests Receiving Emergency Use Authorization, (May 26, 2021)

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Clinical Sensitivity, or Positive Percent Agreement (PPA):

To determine a novel diagnostic test's ability to accurately identify positive subjects, natural clinical samples must be evaluated with the candidate test, and the results compared to data generated with an established comparator diagnostic test. The degree of concordance between positive subjects identified by the candidate test and the established comparator test is reported as the 'Percent Positive Agreement' (PPA), frequently referred to as the clinical sensitivity. For candidate SARS-CoV-2 diagnostic tests, high sensitivity RT-PCR has been established by the FDA as the reference comparator against which clinical performance data must be generated.

Test manufacturers and regulators should be mindful of several potentially confounding factors when considering PPA data. First, a large number of SARS-CoV-2 RT-PCR tests have received Emergency Use Authorization from the FDA, with >100-fold range in analytical sensitivity1. Thus, the specific RT-PCR test(s) used as a comparator in a clinical study may influence the degree of concordance with the candidate test. Second, the size and composition of clinical study cohorts makes it difficult to directly compare clinical performance between tests. Smaller studies, and/or studies with a higher fraction of subjects exhibiting high viral load at the time of testing may not accurately reflect real-world performance. Quanterix has conducted a prospective clinical study enrolling nearly 900 subjects to validate real-world performance of the Simoa SARS-CoV-2 N Protein Antigen Test. This study was 2-10X larger than any other prospective trial conducted for an antigen test authorized for Emergency Use, conferring high confidence that the reported clinical sensitivity accurately reflects test performance. Third, although designated as the established comparator, it has been reported that RT- PCR testing may be susceptible to false negatives early in the infection cycle due to analytical sensitivity limitations2, and susceptible to false positives late in infection due to persistent viral RNA shedding after clinical infection has cleared3. Novel diagnostic tests with performance that is superior to PCR may exhibit apparent "false positives" if detecting viral infection that was missed by molecular testing, or apparent "false negatives" if molecular testing was detecting residual RNA following recovery, rather than viral RNA due to active infection. As new technology platforms enabling high analytical sensitivity such as the Simoa HD-X emerge, it creates new opportunities for the scientific and regulatory community to continuously reassess what test methodology constitutes the "gold standard" against which other tests should be compared.

References:

1. MacKay et al., The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. Nature Biotechnology, Vol 38, September 2020, 1021-1027.
2. Kucirka et al, Variation in False-Negative Rates of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure, Annals of Internal Medicine, 13 May 2020.
3. Surkova E et al., False-positive COVID-19 results: hidden problems and costs. Lancet Respir Med. 2020 Dec;8(12):1167-1168. doi: 10.1016/S2213-2600(20)30453-7. Epub 2020 Sep 29. PMID: 33007240; PMCID: PMC7524437.

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